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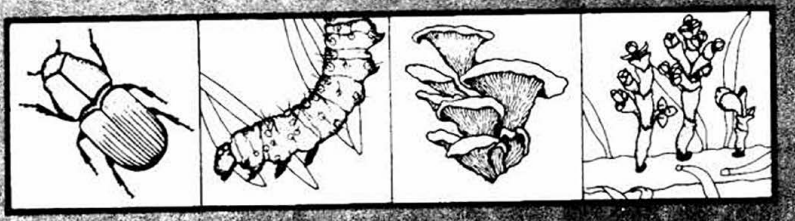

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EVALUATION OF ROOT DISEASES OF CONTAINERIZED CONIFER SEEDLINGS AT THE CHAMPION TIMBERLANDS NURSERY, PLAINS, MONTANA

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ABSTRACT

Investigations of containerized conifer seedling root diseases at the Champion Timberlands Nursery revealed that *Fusarium oxysporum* was most commonly isolated from seed, seedling roots, and styroblock containers. Amount of seed infection varied widely among the seedlots tested, but was usually below 5 percent. Running-water rinses did not reduce amounts of *Fusarium* detected on seed. Douglas-fir and lodgepole pine seedlings with disease symptoms had roots that were much more colonized with *Fusarium* than seedlings without symptoms. *Fusarium* inoculum existed within soil mixes and on root fragments within seedling plugs. The inner walls of styroblocks were also extensively colonized with *Fusarium*, especially at the bottom of the plug. Hot water cleaning and treatment with bleach reduced, but did not eliminate, *Fusarium* within styroblocks. Sufficient inoculum remained to pose potential threats to subsequent crops of seedlings. Resistance to benomyl was not detected with *in vitro* tests, although most *F. oxysporum* isolates displayed some level of resistance to captan, Botran®, and Banrot®.

INTRODUCTION

Containerized seedling production within nurseries in the northern Rocky Mountains has been increasing each year. Several new nurseries have begun production and the number of seedlings produced in established nurseries has increased. Diseases are one of the major limiting factors in the production of conifer seedlings in containers. Conditions within greenhouses used to produce containerized seedlings are often ideal for infection and buildup of pathogenic fungi (James et al. 1983; McCain 1978). As a result, growers must constantly be alert to recognize diseases so that adequate control measures can be instituted.

One of the most troublesome groups of diseases that plague containerized conifer seedling production is root disease caused by *Fusarium* spp. (James 1984a; James 1986a). *Fusarium*-associated root diseases are difficult to control because by the time symptoms appear on seedlings, root infection is extensive and fungicide drenches are only of limited effectiveness (James 1986d; James et al. 1987). Also, many infected seedlings may lack above-ground symptoms (James et al. 1987; James and Gilligan 1988). *Fusarium* inoculum may be introduced into a crop on infected seed (James 1986a; James 1987b), the soil mix (James 1985), and on

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containers that are reused several times (James 1987c; James 1988). Probably the most effective means of reducing damage from *Fusarium*-associated root diseases is by preventing infection through reduction of inoculum or manipulating the soil microbial environment to discriminate against *Fusarium* spp.

Fusarium-associated root disease has been a recurring problem at the Champion Timberlands Nursery in Plains, Montana (James 1984b; James 1986b; James 1986c; James 1986e). Losses have occurred in all species, but seem more substantial in Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco). Efforts to reduce losses from root disease by application of fungicide drenches have largely been ineffective. As a step toward developing more effective control measures, investigations were undertaken to obtain information regarding behavior of *Fusarium* spp. associated with root diseases at the nursery.

MATERIALS AND METHODS

Seed Colonization

Effects of standard running-water rinse treatments used at the nursery on occurrence of fungi on and within Douglas-fir seeds were evaluated. Five commonly used seedlots (table 1; all tables are located in the Appendix) were assayed. Treated seed underwent running-water treatment for 48 hours preceeding stratification (1-2 degrees C for 28 days). Untreated seeds were selected at random from bulk and assayed directly (without stratification). Seeds were placed on a medium selective for *Fusarium* spp. (Komada 1975) and incubated at about 26 degrees C for 7 days under a regime of cool, diurnal, fluorescent light. Numbers of seed colonized by selected groups of fungi were tallied at the end of the incubation period. One hundred seeds per lot were thus assayed.

Fifty seeds per lot were also aseptically dissected to determine occurrence of fungi within the endosperm. Seedcoats were carefully removed and the endosperm placed on Komada's medium and incubated as described above. Numbers of excised endosperms colonized by selected groups of fungi were tallied.

Seedling/Soil Mix Colonization

Ten each containerized Douglas-fir, ponderosa pine (*Pinus ponderosa* Laws.) and lodgepole pine (*Pinus contorta* Dougl.) were selected from the 1986 crop toward the end of the growing cycle. Five seedlings of each species displayed typical root disease symptoms including foliar chlorosis and needle tip dieback (James 1984c; James 1984d; James 1986e). However, only two of the selected seedlings were dead. Five of the seedlings did not have noticeable above-ground disease symptoms. Selected seedlings were rated for severity of their disease symptoms using an 8-point numerical scale developed for containerized seedlings (table 2). Their crown length (from the groundline to the tip of the terminal bud) was also measured.

Root systems of these seedlings were dissected for determination of amount of colonization by *Fusarium* spp. following surface sterilization in 10 percent aqueous sodium hypochlorite and rinsing with sterile distilled water. Ten lateral roots were selected from each root system (if available, i. e., some of the seedlings with severe root decay did not have 10 roots available. In these cases, as many roots as possible were obtained for the sample). Selected roots were severed from the main taproot and pieces approximately 3-5 mm in length were aseptically severed from the tip and the point of attachment with the taproot (joint). Severed pieces were placed on Komada's medium and incubated as described above. Taproots of each seedling were cut into 10 segments approximately 3-5 mm in length and placed on Komada's medium. Number of root tips and joints and number of taproot pieces and estimated length of the taproot colonized with *Fusarium* were determined.

Soil mix, within which seedlings were growing, was collected and sieved (8 mesh -2.4 mm screen) to remove fragments of roots and large pieces of organic material. Pieces of root fragments and organic matter (which were a natural portion of the soil mix) were placed on Komada's medium and incubated as described above. The fine soil mix that had been passed through the sieve was ground with a mortar and pestle to a fine powder. This fine soil underwent standard soil dilution analysis (James and Gilligan 1986) to estimate number of propagules (colony-forming units) per gram. This estimate would give an approximation of the amount of *Fusarium* inoculum found loose in the soil.

Styroblock Colonization

Prior to lifting the fall 1986 crop, 10 styroblocks, each containing 160 seedlings, from three seedlots (designated 18, 19, and 22), were selected for analysis of colonization by *Fusarium*. Selected styroblocks had been used to grow from 1-7 previous crops of seedlings. Within each of the selected styroblocks, five cells were chosen which contained seedlings with root disease symptoms manifested as needletip dieback or needle necrosis (James 1984c; James 1984d), and five which contained nondiseased (asymptomatic) seedlings. Each of the selected seedlings was rated for severity of root disease symptoms (table 1); their height from the groundline to the tip of the terminal bud and caliper just above the groundline were also measured.

After seedling extraction, pieces of styroblock were aseptically cut from each sample cell. Four pieces were cut at about 5 cm from the top of each cell (one in each cardinal direction) with a sterile scalpel and placed (inside surface down) on Komada's medium. Plates were incubated as described above and percentage of sampled cells and styroblock pieces colonized by *Fusarium* and *Trichoderma*, common competitors and antagonists of *Fusarium* (Papavizas 1985), were calculated.

Sample styroblocks were then kept over winter in fallow greenhouses, as is standard at the nursery. In the spring, styroblocks were cleaned using high pressure hot water containing Saniclean® followed by dipping in a 10 percent bleach solution. Following cleaning, the same cells that were originally sampled were resampled at about 5 cm from their top and at their very bottom. Four styroblock pieces were extracted from each of these locations per cell. Pieces were again placed on Komada's medium and incubated as described previously. At the same time, fragments of seedling roots that persisted in cleaned styroblocks were placed on Komada's medium for isolation of *Fusarium*. Colonization percentages of styroblock samples were calculated for both *Fusarium* and *Trichoderma*.

Selected *Fusarium* isolates from seed, seedling roots, soil mixes and styroblocks were transferred to potato dextrose agar (PDA) and carnation leaf agar and identified using the taxonomic guide of Nelson et al. (1983).

Fungicide Resistance

As indicated previously, growers have had problems controlling *Fusarium* root diseases with fungicide drenches once disease symptoms appeared on seedlings. They were concerned that perhaps resident populations of *Fusarium* had developed resistance to commonly used fungicides. Therefore, investigations were conducted to evaluate responses of several isolates of *Fusarium* from the nursery to a low concentration of captan, Botran (dichloran), Benlate® (benomyl), and Banrot®. Responses of selected isolates were evaluated with *in vitro* tests with PDA amended with 5 ug/ml (active ingredient) of the fungicides using procedures developed to assess fungicide resistance of *Botrytis cinerea* (Gillman and James 1980; James and Gilligan 1985). Test isolates were grown on standard PDA for 10 days and 5 mm plugs of mycelium from the advancing margin of colonies were placed on fungicide-amended agar or unamended PDA which served as controls. Plates were incubated at about 22 degrees C under cool fluorescent light and linear growth measured at 2-day intervals. After 14 days

the experiment was terminated; growth on fungicide-amended media was expressed as percentages of growth on unamended media.

RESULTS AND DISCUSSION

Occurrence of selected fungi including *Fusarium* on tested Douglas-fir seedlots is summarized for seedcoats and endosperms in tables 3 and 4, respectively. Levels of *Fusarium* were well within those normally encountered for Douglas-fir within northern Rocky Mountain nurseries (James 1987b). Amounts varied widely among the nine tested seedlots and levels occurring within seed endosperms were generally less than those encountered on seedcoats. Other fungi were also commonly isolated from tested seedlots. Most of these, including *Trichoderma*, *Penicillium*, *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Mucor*, were most likely saprophytic and not important in the degredation of seed quality. Probable pathogens included *Fusarium*, *Botrytis*, and *Cylindrocarpon*. In this evaluation, running-water rinses did not reduce amounts of *Fusarium* on either seedcoats or within seed endosperms (tables 3 and 4). Other fungi, such as *Penicillium* and *Cylindrocarpon* were likewise not reduced by this treatment. On the other hand, levels of *Aureobasidium* and *Cladosporium* were greatly reduced by running-water treatments. Most previous experience (James 1987a; James 1987b; James and Genz 1981) indicated that running water rinses reduce amounts of all fungi, including pathogens such as *Fusarium*, on seed. However, such rinses are most effective if seed is agitated during the process so that fungal propagules can be dislodged from seedcoats (James 1987a; James 1987b). It is unclear why treatments at the Champion Nursery failed to reduce levels of most fungi on seed. However, *Fusarium* levels, even on treated seed, were below those expected to cause severe disease problems.

Significant differences in seedling crown length were not detected between seedlings displaying root disease symptoms and those without symptoms (table 5), although the trend was for reduced height of diseased seedlings for all three species. Although several nondiseased seedlings had roots infected with *Fusarium*, Douglas-fir and lodgepole pine with disease symptoms had much more extensive root colonization by *Fusarium* (table 5). On the other hand, nondiseased ponderosa pine seedlings had roots that were as extensively colonized by *Fusarium* as seedlings with disease symptoms. This confirms other recent findings (James and Gilligan 1988) that containerized ponderosa pine seedlings are often extensively colonized with *Fusarium* even though they lack any disease symptoms. Investigations at the Champion Nursery indicated that *Fusarium* more commonly colonized root tips, although the taproot was often extensively colonized, especially on seedlings with disease symptoms (table 5). These investigations also found that fragments of roots and loose pieces of soil mix often harbored *Fusarium*. Populations of *Fusarium* within soil mixes were very high in cells with diseased seedlings, but much lower in cells with nondiseased seedlings (table 5). This would indicate that under disease conditions which were conducive for pathogens, these fusaria readily grew and reproduced. On the other hand, it is likely that where disease does not occur, some environmental conditions are suppressive to development of *Fusarium*. Although these conditions may be related to the microbial environment (Hoitink and Fahy 1986; Papavizas 1985), the interrelationships at work in containerized conifer seedling growing environments are unknown.

Results from investigating colonization of styroblocks with *Fusarium* indicate that containers which have been reused for several crops are probably important sources of *Fusarium* inoculum (tables 6-8). It appeared that cleaning styroblocks after use reduced amounts of *Fusarium* at least in the upper parts of cells (table 6). However, populations were not eliminated and those at the bottom of cells remained very high even after cleaning. They were definitely high enough to provide an important hazard for the succeeding crop of seedlings. Our results indicated that percentage of cells colonized and intensity of this colonization were not related to the number of times styroblocks had been used (tables 6 & 7). Even after being used only once, styroblocks had high populations of *Fusarium*. These remained high through several additional seedling cycles. Abundance of *Fusarium* was higher within cells which had produced diseased seedlings than those that had nondiseased

seedlings (table 8). It is likely that under disease conditions, fusaria greatly increases within the soil mix (table 5) and grows throughout the inner surface of the styroblock cell.

Similar to *Fusarium* spp., levels of *Trichoderma* on styroblocks were reduced by cleaning, although high levels persisted, especially at the bottom of cells (tables 9 and 10). Since *Trichoderma* spp. are beneficial because of their potential to biologically control *Fusarium* (Papavizas 1985), it would be desirable if higher populations of these organisms would persist on styroblocks at the expense of *Fusarium*. However, their levels were fairly similar to those of *Fusarium* on the sampled styroblocks (compare tables 6 and 9 with 7 and 10). Although *Trichoderma* levels were somewhat higher in cells with nondiseased seedlings (table 11), these levels were not statistically different from those cells with diseased seedlings. Therefore, at least in this study, differences between diseased and nondiseased seedlings could not be explained on the basis of relative abundance of one group of antagonists (*Trichoderma*) within the styroblock environment. It is likely that disease occurrence is a multi-conditional response to many different factors, some of which may be the makeup and abundance of antagonistic microbes (Hoitink and Fahy 1986).

Relationships between intensity of root disease symptoms and seedling height and caliper (table 12), indicated that, in general, diseased Douglas-fir seedlings were smaller than nondiseased seedlings of the same age. Regressions comparing seedling heights with disease ratings produced generally low coefficients of determination (0.04 to 0.54, depending on seedlot). This would indicate that although seedling growth may have been affected by extent of *Fusarium* root disease, other factors may also be important.

Of the more than 50 isolates of *Fusarium* obtained from seed, seedling roots, soil mixes and styroblocks at the nursery, more than 90 percent were *F. oxysporum* Schlect. Two other species, *F. acuminatum* Ell. & Ev. and *F. sambucinum* Fuckel, were isolated infrequently.

From the limited evaluation of fungicide effects on isolates of *F. oxysporum* obtained from the Champion Nursery (table 13), it appeared that most were more resistant to captan and Botran® than to either benomyl or Banrot®. Four of the 10 isolates tested did not grow at all on benomyl, indicating that this fungicide was probably the most effective of those used at the nursery. Therefore, it appears that the failure of benomyl to adequately control *Fusarium* root disease was not due to development of fungal strains resistant to the fungicide. Other factors such as problems with fungicide penetration to roots of infected seedlings and persistance of inoculum within styroblocks probably accounted for high disease incidence despite extensive applications of several fungicides.

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APPENDIX

Table 1.--Douglas-fir seedlots assayed for colonization by fungi from the Champion Timberlands Nursery, Plains, Montana.

Seedlot number	Designation	Location	Treatment
1	80/9-12-16	Ashby Creek	Untreated
2	80/9-12-16	Ashby Creek	Stratified & running-water rinse
3	80/18-12-16	Wallace Creek	Untreated
4	80/18-12-16	Wallace Creek	Stratified & running-water rinse
5	80/29-10-17	Rock Creek	Untreated
6	80/29-10-17	Rock Creek	Stratified & running-water rinse
7	85/13-12-16	Ashby Creek	Untreated
8	85/13-12-16	Ashby Creek	Stratified & running-water rinse
9	85/11-14-9 *1/*	Stonewall Creek	Untreated

1/ Treated seedlot 82/11-14-9 was unavailable.

Table 2.--Numerical rating system for severity of above-ground root disease symptoms for containerized conifer seedlings.

Rating	Description
0	No symptoms; seedling crown entirely green.
1	Seedling with slight needle tip dieback, particularly concentrated on ten upper whorls of needles.
2	Seedling with lower whorl of needles partially or completely necrotic; seedling upright.
3	Seedling with needle tip dieback affecting at least one-half of the crown.
4	Seedling with one-half of its crown with necrotic foliage (upper or lower); seedling upright.
5	Seedling with one-half of its crown with necrotic foliage (upper or lower); seedling bent over.
6	Seedling with three-quarters of its crown with necrotic foliage; seedling may be upright or bent over.
7	Seedling with its entire crown necrotic; seedling may be upright or bent over.

Table 3.–Occurrence of selected fungi on seedcoats of Douglas-fir seed from the Champion Timberlands Nursery. 1/

	SEEDLOTS 2/									AVERAGES		
Fungi	1	2	3	4	5	6	7	8	9	All untreated seedlots 3/	All treated seedlots 3/	All seedlots
Alternaria	1.3	0	0	0	1.3	0	1.3	0	0	0.8 NS	0 NS	0.4
Aureobasidium	32.7	4.0	12.7	0.7	39.3	0	25.3	2.7	18.0	25.6 *	1.8 *	15.0
Botrytis	0.7	0	0.7	0	0.7	1.3	0.7	0.7	0	0.5 NS	0.5 NS	0.5
Cladosporium	19.3	0	16.7	0	11.3	0	2.0	0	2.0	10.2 *	0 *	5.7
Cylindrocarpon	0	2.7	0.7	0	0	0	2.0	0	0	0.5 NS	0.7 NS	0.6
Fusarium	0.7	2.7	0.7	2.0	4.0	6.0	0.7	2.0	3.3	1.9 NS	3.2 NS	2.4
Mucor	0	0.7	0	0	0	0	1.3	0	0	0.3 NS	0.2 NS	0.2
Penicillium	68.7	94.0	94.0	100.0	64.0	100.0	92.7	99.3	58.0	75.5 *	98.3 *	85.6
Trichoderma	20.7	12.0	6.0	8.0	3.3	4.7	22.0	11.3	2.7	10.9 NS	9.0 NS	10.1
None	6.7	1.3	1.3	0	4.0	0	0	0.7	24.0	7.2 *	0.5 *	4.2

1/ Figures in table are percentages of sampled seed (150 per seedlot) colonized with appropriate organisms when incubated on Komada's medium for 7 days at 26 degrees C.

2/ See table 1 for seedlot descriptions.

3/ NS = means not statistically different using standard "t" tests comparing treated vs. untreated seed.

* = means statistically different (P=0.05) using standard "t" tests comparing treated vs. untreated seed.

Table 4.--Occurrence of selected fungi on endosperms of Douglas-fir seed from the Champion Timberlands Nursery. 1/

	SEEDLOTS 2/									AVERAGES		
Fungi	1	2	3	4	5	6	7	8	9	All untreated seedlots 3/	All treated seedlots 3/	All seedlots
Alternaria	2.0	0	8.0	0	4.0	4.0	4.0	4.0	0	3.6 NS	2.0 NS	2.9
Aureobasidium	16.0	2.0	0	0	18.0	6.0	44.0	0	2.0	16.0 *	2.0 *	9.8
Botrytis	0	0	2.0	0	0	0	2.0	0	0	0.8 NS	0 NS	0.4
Cladosporium	2.0	0	14.0	2.0	2.0	0	0	2.0	0	3.6 NS	1.0 NS	2.4
Cylindrocarpon	0	0	0	2.0	0	2.0	0	0	0	0 NS	1.0 NS	0.4
Fusarium	2.0	0	2.0	2.0	4.0	6.0	0	2.0	0	1.6 NS	2.5 NS	2.0
Mucor	0	2.0	0	0	0	0	0	0	0	0 NS	0.5 NS	0.2
Penicillium	14.0	74.0	48.0	78.0	8.0	90.0	28.0	100.0	6.0	20.8 *	85.5 *	49.5
Trichoderma	0	0	0	0	0	0	2.0	0	0	0.4 NS	0 NS	0.2
None	62.0	22.0	26.0	22.0	64.0	4.0	32.0	0	90.0	54.8 *	12.0 *	35.8

1/ Figures in table are percentages of endosperms (50 per seedlot) colonized with appropriate organisms when incubated on Komada's medium for 7 days at 26 degrees C.

2/ See table 1 for seedlot descriptions.

3/ NS = means not statistically different using standard "t" tests comparing treated vs. untreated seed.

* = means statistically different (P=0.05) using standard "t" tests comparing treated vs. untreated seed.

Table 5.–Occurrence of Fusarium spp. within container soil mixes and on Douglas-fir, ponderosa pine, and lodgepole pine root systems at the Champion Timberlands Nursery, Plains, Montana.

Species	Root Disease Symptoms	Avg. Dis. Rating 1/ 7/	Avg. Crown Length (mm) 7/	Average Percent Root Colonization					Soil Mix Colonization		Colony-Forming Units per gm of Soil Mix 7/
				Laterals		Taproot		Entire Root System 4/ 7/	Percent Root Fragments Colon. 5/ 7/	Percent Soil Mix Pieces Colon. 6/ 7/	
				Tips	Joints	Sample 2/	Colon. 3/				
Douglas-fir	Present	4.8 *	165.4 NS	100	66	92	84.0	84.5 *	91.6 *	93.9 NS	17,548 *
	Absent	0 *	195.2 NS	50	4	6	3.3	9.0 *	38.6 *	57.8 NS	1,680 *
	Both	2.4	180.3	79	35	49	40.1	44.4	65.1	77.3	9,614
Ponderosa pine	Present	3.4 *	208.2 NS	52	14	38	36.1	35.6 NS	33.6 NS	20.2 NS	4,752 *
	Absent	0.8 *	213.2 NS	78	38	52	55.8	55.9 NS	33.3 NS	38.5 NS	528 *
	Both	2.1	210.7	65	26	45	48.4	47.6	33.5	29.5	2,640
Lodgepole pine	Present	3.2 *	224.0 NS	90	20	70	59.5	66.7 *	70.5 *	74.1 *	6,484 *
	Absent	0.4 *	253.2 NS	62	14	24	16.1	21.0 *	19.5 *	5.5 *	296 *
	Both	1.8	238.6	76	17	47	38.8	43.3	43.8	38.6	3,390
All species	Present	4.1 *	199.2 NS	80.7	33.3	66.7	61.4	60.6 *	66.9 *	63.2 *	9,595 *
	Absent	0.4 *	220.5 NS	66.0	18.7	27.3	23.6	27.4 *	30.3 *	34.8 *	835 *
	Both	2.2	209.9	73.3	26.0	47.0	42.5	44.0	48.6	49.0	5,215

1/ See table 2 for description of ratings.

2/ Percent of taproot pieces sampled which were colonized with Fusarium spp.

3/ Percent of taproot length colonized with Fusarium spp.

4/ A composite estimate of the percent of the root system colonized with Fusarium spp. Estimate is based on the percentage of root pieces sampled which were colonized with Fusarium.

5/ Unattached root pieces located with the soil mix.

6/ Includes pieces of vermiculite and peat moss not passing through an 8 mesh (2.4 mm) soil screen.

7/ NS = means not statistically different using standard "t" tests comparing seedlings with root disease symptoms and those without symptoms.

* = means statistically different ($P=0.05$) using standard "t" tests comparing seedlings with root disease symptoms and those without symptoms.

Table 6.–Occurrence of *Fusarium* spp. on styroblock containers from the Champion Timberlands Nursery, Plains, Montana.

		Percent of Cells Colonized with *Fusarium* spp.		
			After Cleaning	
No. Years Used	No. Cells Sampled	Prior to Cleaning (Top*)	Top *	Bottom
1	20	25.0 **	10.0 **	70.0
3	40	50.0 **	7.5 **	62.5
6	20	70.0 **	10.0 **	75.0
7	20	25.0 NS	25.0 NS	55.0
Totals	100	44.0 **	12.0 **	65.0

* Location of sample: Top = within 5 cm of cell top
Bottom = at bottom of cell.

** means of percent of cells (top only) colonized prior to and after cleaning are significantly different (P=0.05) using standard "t" tests.

NS means of percent of cells (top only) colonized prior to cleaning and after cleaning are not significantly different using standard "t" tests. All percentages underwent arc-sin conversions prior to analysis.

Table 7.--Colonization intensity of styroblock containers by *Fusarium* spp. at the Champion Timberlands Nursery, Plains, Montana.

		Colonization Intensity by *Fusarium* spp. *		
			After Cleaning	
No. Years Used	No. Cells Sampled	Prior to Cleaning (Top **)	Top **	Bottom **
1	20	10.0	5.0	41.3
3	40	17.5	6.2	29.4
6	20	38.8	10.0	38.8
7	20	7.5	25.0	28.8
Totals	100	18.8	7.5	34.2

* Based on percent of styrofoam pieces colonzied (four pieces selected per cell in each location during each sampling period).

**Location of sample: Top = within 5 cm of cell top
Bottom = at bottom of cell.

Table 8.--Colonization of styrofoam cells containing diseased and nondiseased Douglas-fir seedlings with *Fusarium* spp. at the Champion Timberlands Nursery, Plains, Montana.

	No. Samples	% Cells Colonized *	Colonization Intensity *
Diseased seedlings	150	50.0 **	27.2
Nondiseased seedlings	150	30.6 **	13.2
Totals	300	40.3	20.2

* Aggregated data for samples taken prior to and following cleaning at both top and bottom of cells. Colonization intensity is based on percent of styrofoam pieces colonized (four pieces selected per cell in each location during each sampling period).

** Means of percent of cells containing diseased and nondiseased seedlings which were colonized with *Fusarium* spp. that were significantly different ($P=0.05$) using a standard "t" test. All percentages underwent arc-sin transformations prior to analysis.

Table 9.--Occurrence of *Trichoderma* spp. on styroblock containers from the Champion Timberlands Nursery, Plains, Montana.

		Percent of Cells Colonized with *Trichoderma* spp.		
			After Cleaning	
No. Years Used	No. Cells Sampled	Prior to Cleaning (Top *)	Top *	Bottom *
1	20	45.0 **	10.0 **	55.0
3	40	50.0 **	5.0 **	45.0
6	20	45.0 **	20.0 **	40.0
7	20	75.0 **	25.0 **	40.0
Totals	100	53.0 **	13.0 **	45.0

* Location of sample: Top = within 5 cm of cell top
Bottom = at bottom of cell.

** Means of percent of cells (top only) colonzied prior to and after cleaning are significantly different (P=0.05) using standard "t" tests. All percentages underwent arc-sin conversions prior to analysis.

Table 10.--Colonization intensity of styroblock containers by *Trichoderma* spp. at the Champion Timberlands Nursery, Plains, Montana.

		Colonization Intensity by *Trichoderma* spp. *		
			After Cleaning	
No. Years Used	No. Cells Sampled	Prior to Cleaning (Top **)	Top **	Bottom **
1	20	13.8	5.0	22.5
3	40	22.5	2.5	21.3
6	20	15.0	10.0	15.0
7	20	33.8	17.5	13.8
Totals	100	21.5	7.5	18.8

* Based on percent of styrofoam pieces colonzied (four pieces selected per cell in each location during each sampling period).

**Location of sample: Top = within 5 cm of cell top
Bottom = at bottom of cell.

Table 11.—Colonization of styrofoam cells containing diseased and nondiseased Douglas-fir seedlings with *Trichoderma* spp. at the Champion Timberlands Nursery, Plains, Montana.

	No. Samples	% Cells Colonized *	Colonization Intensity *NS*
Diseased seedlings	150	34.0 NS	13.5
Nondiseased seedlings	150	40.0 NS	18.2
Totals	300	37.0	15.9

* Aggregated data for samples taken prior to and following cleaning at both top and bottom of cells. Colonization intensity is based on percent of styrofoam pieces colonized (four pieces selected per cell in each location during each sampling period).

NS Means of percent of cells containing diseased and nondiseased seedlings which colonized with *Trichoderma* spp. were not significantly different (P=0.05) using a standard "t" test. All percentages underwent arc-sin transformations prior to analysis.

Table 12.--Relationships between intensity of root disease symptoms and seedling height and caliper of containerized Douglas-fir seedlings extracted from sampled styroblock containers.

	Nondiseased Seedlings			Diseased Seedlings		
Seedlot	Avg. Dis. Rating*	Avg. Hgt. (mm)*	Avg. Caliper (mm)*	Avg. Dis. Rating*	Avg. Hgt. (mm)*	Avg. Cal. (mm)
18	0.0	154.7	3.0	4.8	153.6	2.4
19	0.6	138.0	3.2	3.8	116.2	2.5
22	0.1	137.2	2.9	4.7	109.7	2.2
All lots	0.18	140.8	3.0	4.52	119.8	2.3

* Coefficients of determination (R^2) comparing disease ratings with seedling heights using simple linear regressions were:

Seedlot	R^2	Regression Equation
18	0.04	Y = 155.3 - 0.5 x
19	0.54	Y = 143.5 - 7.5 x
22	0.34	Y = 137.8 - 6.0 x

Table 13.--Effects of selected fungicides on the radial growth of isolates of *Fusarium oxysporum*. 1/

			Percent of Control			
Isolate	Host 2/	Control growth (mm/day)	Banrot	Benlate	Botran	Captan
87-2 A	DF	8.1	37.0 A 3/	1.2 A	35.8 A	43.2 A
87-2 B	DF	9.5	35.8 A	1.6 A	32.6 A	37.9 A
87-2 C	DF	9.3	12.9 B	3.3 A	39.8 A	44.1 A
87-2 D	LP	8.1	12.3 B	0.2 B	35.8 A	24.7 A
87-2 E	PP	8.2	18.3 B	2.1 A	43.9 A	45.1 A
87-3 A	LP	8.2	15.8 B	0 B	40.2 A	48.8 A
87-3 B	LP	9.6	13.5 B	0 B	38.5 A	38.5 A
87-3 C	PP	7.9	8.9 B	0.8 B	38.0 A	40.5 A
87-3 D	PP	9.1	14.3 B	0 B	27.5 A	36.3 A
87-3 E	DF	9.6	9.4 B	0 B	31.2 A	37.5 A
Averages	--	8.76	17.8	0.9	36.2	39.6

1/ Based on linear growth of isolates on PDA amended with 5 mg/ml (active ingredient) of the fungicide.

2/ DF = Douglas-fir; LP = lodgepole pine; PP = ponderosa pine

3/ Within each row, means followed by the same capital letter were not significantly different (P=0.05) using Tukey's Multiple Range Comparison Test.